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ABSTRACT

Background: micronutrients (MNs) in the diet are linked to arthritis, but the causal relationships and dose-dependent effects remain unclear.

Methods: using the National Health and Nutrition Examination Survey (NHANES) and genome-wide association studies (GWAS) databases, multivariate logistic regression, restricted cubic splines (RCS), and Mendelian randomization (MR) were applied to analyze MNs' impact on arthritis.

Results: multivariate logistic regression identified six MNs significantly associated with arthritis: calcium (OR = 0.74 [95 % CI, 0.60, 0.92] p = 0.013), magnesium (OR = 0.74 [95 % CI, 0.62, 0.89] p = 0.003), selenium (OR = 0.62 [95 % CI, 0.50, 0.76] p = 0.0001), folic acid (OR = 0.76 [95 % CI, 0.61, 0.94] p = 0.018), vitamin E (OR = 0.77 [95 % CI, 0.64, 0.92] p = 0.01), vitamin B6 (OR = 0.64 [95 % CI, 0.53, 0.78] p = 0.0001). RCS analysis revealed significant non-linear relationships for vitamin B6, vitamin E, folic acid, and selenium ($p < 0.05$). MR analysis only confirmed causal associations in specific MNs and subtypes of arthritis: calcium and rheumatoid arthritis (OR = 0.648 [95 % CI, 0.436, 0.962] p = 0.031), vitamin B12 and monoarthritis (OR = 2.876 [95 % CI, 1.240, 6.667] p = 0.014), potassium and pyogenic arthritis (OR = 2.124 [95 % CI, 1.003, 4.497] p = 0.049), vitamin B6 and pyogenic arthritis (OR = 1.744 [95 % CI, 1.034, 2.940] p = 0.037), vitamin B12 and other arthritis (OR = 1.414 [95 % CI, 1.035, 1.932] p = 0.03).

Conclusions: this study reveals the causal relationship between specific MNs and certain arthritis subtypes, while also observing dose-dependent associations between other MNs and arthritis. These findings provide valuable insights for dietary interventions and preventive strategies targeting arthritis.

Keywords: Micronutrients. Arthritis. NHANES. Mendelian randomization.

RESUMEN

Antecedentes: los micronutrientes (MN) de la dieta están relacionados con la artritis, pero las relaciones causales y los efectos dependientes de la dosis siguen sin aclararse.

Métodos: utilizando la Encuesta Nacional de Examen de Salud y Nutrición (NHANES) y bases de datos de estudios de asociación del genoma completo (GWAS), se aplicaron la regresión logística multivariante, *splines* cúbicos restringidos (RCS) y la aleatorización mendeliana (MR) para analizar el impacto de los MN en la artritis.

Resultados: la regresión logística multivariada identificó seis MN significativamente asociados con la artritis: calcio (OR = 0,74 [IC 95 %, 0,60, 0,92] p = 0,013), magnesio (OR = 0,74 [IC 95 %, 0,62, 0,89] p = 0,003), selenio (OR = 0,62 [IC 95 %, 0,50, 0,76] p = 0,0001), ácido fólico (OR = 0,76 [IC 95 %, 0,61, 0,94] p = 0,018), vitamina E (OR = 0,77 [IC 95 %, 0,64, 0,92] p = 0,01), vitamina B6 (OR = 0,64 [IC 95 %, 0,53, 0,78] p = 0,0001). El análisis RCS reveló relaciones no lineales significativas para la vitamina B6, la vitamina E, el ácido fólico y el selenio ($p < 0,05$). El análisis de MR solo confirmó asociaciones causales entre MN específicos y subtipos de artritis: calcio y artritis reumatoide (OR = 0,648 [IC 95 %, 0,436, 0,962] p = 0,031), vitamina B12 y monoartritis (OR = 2,876 [IC 95 %, 1,240, 6,667] p = 0,014), potasio y artritis piógena (OR = 2,124 [IC 95 %, 1,003, 4,497] p = 0,049), vitamina B6 y artritis piógena (OR = 1,744 [IC 95 %, 1,034, 2,940] p = 0,037), vitamina B12 y otras artritis (OR = 1,414 [IC 95 %, 1,035, 1,932] p = 0,03),

Conclusiones: este estudio revela asociaciones causales entre MN específicos y subtipos específicos de artritis, al mismo tiempo que observa relaciones dosis-dependientes entre otros MN y la artritis. Estos hallazgos aportan valiosas perspectivas para las intervenciones

dietéticas y estrategias preventivas dirigidas específicamente a la artritis.

Palabras clave: Micronutrientes. Artritis. NHANES. Aleatorización mendeliana.

INTRODUCTION

Arthritis is a common chronic disease that primarily affects joints and muscles, characterized by joint pain, inflammation, and limited mobility (1). It is prevalent among adults and can lead to disability (2). Trace elements and vitamins, collectively known as micronutrients (MNs), are essential for human metabolism. The importance of MNs in common pathologies is recognized by recent research (3). Dietary MNs play a crucial role in the occurrence and development of arthritis, and increasing evidence suggests that differences in micronutrient intake may significantly influence the pathogenesis, treatment response, and remission of synovitis. Randomized clinical trials on the intake of specific MNs or nutrient-rich foods have shown improvements in symptoms, along with regulation of both pro-inflammatory and anti-inflammatory mediators (4). However, there is relatively little research on the causal relationship between different MNs and arthritis.

The National Health and Nutrition Examination Survey (NHANES) is an epidemiological survey administered by the National Center for Health Investigation, which is updated every two years and provides health and nutrition data of the United States population (5). In recent years, more and more studies have been conducted using nutrition survey data. One study showed that zinc and vitamin D are currently the most well-studied and promising candidates for intervention to treat autoimmune diseases (6), and another study showed a beneficial relationship

between increased dietary vitamin E intake and reduced risk of all-cause death in RA patients (7). These research results provide ideas for our research, but how to verify our findings has become a difficult problem.

Mendelian randomization (MR) is a method used to evaluate the causal relationship between modifiable exposure or risk factors and clinically relevant outcomes (8), which is favored by a large number of researchers because it can partially replace Randomized controlled trials studies (9). One study identified associations between 14 MNs and 22 cancer outcomes through MR Analysis (10). Another study used MR Analysis to explore the causal relationship between MNs and uric acid levels and gout risk (11). Analysis of the effect of dietary MNs on arthritis in combination with NHANES and verification by MR Method can greatly enhance the credibility of this study.

We hypothesized that specific MNs exhibit dose-dependent effects on arthritis incidence, with optimal doses providing the greatest protective benefits. This study utilized NHANES data and MR analysis to identify diet-related MNs causally linked to arthritis and assess the effects of their varying doses on disease development. These findings provide a foundation for drug development and future interventions tailored to micronutrient levels.

METHODS

The design of the study is summarized in figure 1.

Study population in NHANES

This study used the NHANES dataset from 2015 to 2018, which included a total of 19,225 people. Incomplete, unreliable, or uncertain data were screened and 9,289 eligible participants were selected (Suppl. Table I). The NHANES study was approved by the National Ethical Review Board for Health Statistics, and this study used publicly available data, so no additional institutional review board approval was required.

Assessment of arthritis in NHANES

The definition of arthritis is based on items in the medical condition questionnaire completed in personal interviews, SAS Label “Doctor ever said you had arthritis”. In this study, relevant data sets from 2015 to 2018 were collected and analyzed as outcomes.

Definition of MNs in NHANES

To investigate the relationship between dietary MNs and arthritis, we selected 14 relevant factors, including “carotene, calcium, copper, folic acid, iron, potassium, selenium, magnesium, vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin D, and vitamin E”. The data were sourced from the NHANES 2015-2018 dietary interview questionnaire item “Dietary interview - Total nutrient intakes, first day”.

Covariates used in NHANES

Covariates include age, sex, race, education level, body mass index (BMI), alcohol consumption and smoking. Age and BMI were treated as continuous variables. The categories of covariables are as follows: gender (female, male), race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, non-Hispanic Asian, other race), education level (below 9th grade, 9-11 grades, high school graduation/GED or equivalent, some college or AA degree, college graduate or above), had at least 12 alcohol drinks/1 year (yes, no), smoked at least 100 cigarettes in life (yes, no).

Data used for MR analysis

We obtained data on 14 MNs from the publicly available GWAS database (https://gwas.mrcieu.ac.uk/), including “carotene, calcium, copper, folic acid, iron, potassium, selenium, magnesium, vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin D, and vitamin E” (Suppl. Table II). We

selected this data as exposure factors, defining single nucleotide polymorphisms (SNPs) as instrumental variables (IVs). The tool variable SNPs were analyzed for correlation with p1 = 5e-6 and p2 = 5e-6, using kb = 10,000 and r^2 = 0.001 to remove SNPs in linkage disequilibrium. Weak IVs were excluded based on F-test values < 10 (Suppl. Table III).

We obtained genetic data related to 13 types of arthritis from the FinnGen GWAS, including "other/unspecified seropositive rheumatoid arthritis, seropositive rheumatoid arthritis, rheumatoid arthritis, other (seronegative) rheumatoid arthritis, juvenile arthritis, other arthritis, seronegative rheumatoid arthritis, other/unspecified rheumatoid arthritis, polyarthritis, unspecified, monoarthritis, not elsewhere classified, pyogenic arthritis, chronic crepitant synovitis/bursitis of hand and wrist/periarthritis of wrist" (Suppl. Table IV). All these data were from European population, with a total sample size of 4,726,418, including 7,167 samples of arthritis, as the outcome of this study.

Statistical analysis

According to the NHANES analysis guidelines, sample weights, stratification, and primary sampling units were used to account for the complex survey design. Continuous variables are presented as means and standard deviations (SD), while categorical variables are presented as frequencies and percentages. We used t-tests and chi-square tests to compare the variation of continuous and categorical variables, respectively. Multivariable adjusted logistic regression analysis was employed to assess the relationship between 14 MNs and arthritis. Three models were evaluated for covariate adjustment: Model 1 was unadjusted; Model 2 included age, sex, race, education level, BMI, alcohol consumption, and smoking status; Model 3 additionally adjusted for the remaining 13 MNs after excluding the MNs of interest. Participants were categorized into quartiles based on their oral

micronutrient concentrations, with the lowest level representing the reference group (quartile Q1). Results are presented as odds ratios (OR) with 95 % confidence intervals (CI) and *p*-values. We utilized the restricted cubic splines (RCS) method to construct curves for a deeper understanding of the non-linear relationship between MNs and arthritis. Finally, to assess whether there were differential effects across different subgroups, we conducted subgroup analyses. This included dividing the sample into different subgroups based on age, sex, race, education level, BMI, alcohol consumption, and smoking status, and assessing the relationships between variables within these subgroups.

This study employed two-sample MR to investigate the causal relationship between dietary MNs and arthritis. MR analysis was conducted using the "Two Sample MR" R package, applying inverse variance weighting (IVW) to assess the risk relationships between 14 MNs and 13 types of arthritis. We used IVW results as the primary statistical analysis method, selecting results with $p < 0.05$ while excluding results from five statistical methods that showed OR values in different directions. Sensitivity analyses were performed through heterogeneity tests, horizontal pleiotropy tests, leave-one-out (LOO) analysis, and pleiotropy residual sum and outlier (PRESSO) to ensure the reliability of our conclusions. All statistical analyses were conducted using R software (version 4.4.1), with $p < 0.05$ considered statistically significant.

RESULTS

Characteristics of study participants delineated by arthritis diagnosis

The general characteristics of the study population selected from the NHANES database are shown in table I. A total of 9,289 subjects participated in the analysis, including 6,596 subjects without arthritis (normal group) and 2,693 subjects with arthritis (disease group). The

average age of the normal group and the disease group was 45.80 ± 16.94 years and 62.13 ± 13.36 years, respectively ($p < 0.001$). There was a significant difference in gender between the groups, with a higher proportion of males in the disease group (1,560 males, 57.9 %, $p < 0.001$). Compared to the normal group, the disease group had a significantly higher BMI (29.11 ± 6.92 vs. 31.56 ± 7.67, $p < 0.001$). Statistically significant differences were observed between the normal and disease groups in terms of race ($p < 0.001$), educational level ($p < 0.007$), and smoking history ($p < 0.001$), while no statistically significant difference was found in drinking history ($p = 0.977$).

The results of MNs in the diets of subjects from the normal and disease groups are shown in table II. Nine MNs showed statistically significant differences that have a protective effect against arthritis: vitamin B6 ($p = 0.008$), vitamin B12 ($p = 0.016$), vitamin E ($p = 0.013$), calcium ($p = 0.006$), copper ($p = 0.006$), folic acid ($p = 0.005$), iron ($p = 0.042$), magnesium ($p = 0.002$), and selenium ($p < 0.001$).

Dietary MNs in relation to arthritis

We conducted a multivariable-adjusted logistic regression analysis to assess the relationship between dietary MNs and arthritis (Suppl. Table V). The subjects were divided into quartiles based on their oral micronutrient concentrations, with the lowest level serving as the reference group (quartile Q1). In model 1, six MNs were found to be significantly associated with arthritis: calcium (OR = 0.74 [95 % CI, 0.60, 0.92], $p = 0.013$), magnesium (OR = 0.74 [95 % CI, 0.62, 0.89], $p = 0.003$), selenium (OR = 0.62 [95 % CI, 0.50, 0.76], $p = 0.0001$), folic acid (OR = 0.76 [95 % CI, 0.61, 0.94], $p = 0.018$), vitamin E (OR = 0.77 [95 % CI, 0.64, 0.92], $p = 0.01$), and vitamin B6 (OR = 0.64 [95 % CI, 0.53, 0.78], $p = 0.0001$). The effects of these MNs on arthritis seemed to increase with dosage, with the most significant associations observed at the following doses: calcium (1162-8470 mg), magnesium (368-

1654 mg), selenium (101.0-1195.6 mg), folic acid (119-3522 μg), vitamin E (10.92-138.55 μg), and vitamin B6 (2.466-44.751 μg). However, after adjusting for a range of potential confounding factors in models 2 and 3, the significance of these associations was weakened or even disappeared. This suggests that confounding factors may have influenced the significant associations observed in the unadjusted model to some extent. Nevertheless, the results of model 1 still provide us with a preliminary perspective on which MNs may potentially be associated with arthritis, and more methods need to be combined for analysis.

Next, the RCS curve was used to further analyze the nonlinear relationship between MNs and arthritis (Fig. 2). The RCS analysis revealed significant nonlinear relationships between the incidence of arthritis and four MNs: vitamin B6 ($p < 0.001$), vitamin E ($p = 0.019$), folic acid ($p = 0.014$), and selenium ($p = 0.016$). These results indicate that at certain intake levels, these MNs are associated with a lower risk of arthritis. However, it should be noted that these "intake ranges associated with lower risk" are not equivalent to the recommended optimal doses or intervention thresholds. For example, the selenium intake level observed in the RCS analysis (101.0 mg) is far beyond the recommended daily intake (about 55 μg/day), which suggests that this result may reflect more of the association patterns in observational studies rather than actual actionable intake recommendations. Therefore, we suggest comparing these results with the dietary reference intakes (DRIs) to better understand their relevance in practical applications. In addition, calcium, magnesium, iron, copper, and vitamin B12 did not show significant nonlinear relationships with the incidence of arthritis.

Subgroup analysis

We categorized the sample into different subgroups based on age, gender, race, education level, BMI, alcohol consumption, and smoking

status, and conducted subgroup analyses (Fig. 3). The forest plot visually displayed the ORs and their 95 % CI for each subgroup, and the significance of intergroup differences was assessed through the interaction *p*-value. In the subgroup analysis, we found significant associations between race and arthritis (interaction *p*-value = 0.045). Specifically, Mexican Americans (OR = 0.822, 95 % CI: 0.703-0.963, $p = 0.024$), non-Hispanic blacks (OR = 0.887, 95 % CI: 0.829-0.948, $p = 0.002$), and other Hispanics (OR = 0.765, 95 % CI: 0.684-0.857, $p < 0.001$) had a significantly lower risk of arthritis, while no significant differences were observed in other races. Significant intergroup differences were found in education level (interaction *p*-value = 0.022). Specifically, those with 9-11 grades (OR = 0.867, 95 % CI: 0.782-0.961, $p = 0.011$), high school graduates (OR = 0.873, 95 % CI: 0.789-0.966, $p = 0.014$), and some college education (OR = 0.897, 95 % CI: 0.813-0.988, $p = 0.036$) had a lower risk, while no significant associations were found in those with less than 9th grade ($p = 0.057$) or those with a college degree or above ($p = 0.927$). In the BMI stratification, the Q3 group (28.6-33.5) had a significantly lower risk of arthritis (OR = 0.863, 95 % CI: 0.778-0.956, $p = 0.009$), while no statistical differences were observed in other groups (interaction *p*-value = 0.674). Moreover, subgroup analyses for gender, age, alcohol consumption, and smoking did not show significant intergroup differences (all interaction *p*-values > 0.25), suggesting that these factors may have limited effects on moderating the risk of arthritis.

Causal association of dietary MNs and arthritis risk in MR

This study utilized two-sample MR analysis to explore the causal relationships between different MNs in the diet and various types of arthritis. Fourteen MNs and thirteen types of arthritis were analyzed using two-sample MR. We found causal relationships between four MNs and four types of arthritis (Fig. 4A): calcium and rheumatoid arthritis (OR

= 0.648 [95 % CI, 0.436, 0.962] p = 0.031), vitamin B12 and monoarthritis (OR = 2.876 [95 % CI, 1.240, 6.667] p = 0.014), potassium and pyogenic arthritis (OR = 2.124 [95 % CI, 1.003, 4.497] p = 0.049), vitamin B6 and pyogenic arthritis (OR = 1.744 [95 % CI, 1.034, 2.940] p = 0.037), and vitamin B12 and other arthritis (OR = 1.414 [95 % CI, 1.035, 1.932] p = 0.03). By plotting scatter plots, we more directly illustrated the causal relationships between exposure and outcome. Calcium has a protective effect on rheumatoid arthritis; as calcium levels increase, the incidence of rheumatoid arthritis decreases (Fig. 4B). Vitamin B12 promotes monoarthritis; as vitamin B12 levels rise, the incidence of monoarthritis also increases (Fig. 4C). Both potassium and vitamin B6 promote pyogenic arthritis (Fig. 4D and 4E). In other arthritis, higher levels of vitamin B12 are associated with an increased incidence of the disease (Fig. 4F). Heterogeneity test, horizontal pleiotropy test and PRESSO sensitivity analysis were used to ensure the reliability of the conclusions (Table III). A leave-one-out (LOO) plot was also drawn to verify our findings (Suppl. Fig. 1).

DISCUSSION

Our hypothesis was confirmed, as specific MNs were found to be significantly associated with arthritis, and dose-dependent relationships were identified for several of these MNs. In this study, we used nutritional survey data from NHANES 2015-2018 to analyze the relationship between 14 MNs and arthritis, identifying 9 significant MNs: vitamin B6, vitamin B12, vitamin E, calcium, copper, folic acid, iron, magnesium, and selenium. Through multivariable-adjusted logistic regression analysis, we found that 6 MNs (calcium, magnesium, selenium, folic acid, vitamin E, and vitamin B6) were significantly associated with arthritis. The RCS analysis revealed significant nonlinear

relationships between vitamin B6, vitamin E, folic acid, selenium, and the incidence of arthritis, suggesting that these MNs may be associated with a lower risk of arthritis at specific intake levels. Additionally, subgroup analyses indicated significant associations between race, education level, BMI, and arthritis. Finally, combining these findings with two-sample MR analysis, we identified causal relationships between four MNs and four types of arthritis.

Calcium is an essential micronutrient in the human body, closely related to bone growth and development, and significantly associated with the incidence of osteoporotic fractures or fragility fractures. Adequate supplementation of calcium can reduce the occurrence of many bone-related diseases (12). One study indicated that moderate to high dietary calcium intake could decrease the incidence of hip fractures when following a generally healthy diet (13). Unfortunately, dietary calcium deficiency is a global public health issue, with insufficient calcium intake being common across different countries and age groups (14). Research on NHANES data found a significant association between calcium and arthritis, particularly with supplementation amounts between 1162 and 8470 mg, where the effect on reducing arthritis incidence was more pronounced, though no significant nonlinear relationship was observed between the two. MR analysis showed a clear causal relationship between calcium and rheumatoid arthritis, with increasing calcium levels associated with a reduced incidence of the disease. Future research will focus on safe and effective calcium supplementation methods and ways to enhance its absorption.

Vitamin B6 is crucial for neurological health, as it aids in the synthesis of neurotransmitters related to mood regulation and supports immune function by enhancing the immune response through lymphocyte production. One study indicated that high doses of vitamin B6 can reduce sensory hyperreactivity and other aspects of sensory responsiveness (15). Additionally, vitamin B6 contributes to

cardiovascular health by lowering the risk of heart disease through the regulation of homocysteine levels and supporting red blood cell production, thus promoting blood health. It also plays a role in energy metabolism and may alleviate nausea during pregnancy. Adequate intake of vitamin B6 can reduce the risk of certain cancers and promote eye health. This study found that vitamin B6 has a significant effect on arthritis, with a daily dosage of 2.466 μg showing a notable nonlinear relationship. However, exceeding this dosage did not provide additional protective effects and could even be harmful for certain types of arthritis, particularly in pyogenic arthritis, where excessive vitamin B6 intake may promote the incidence of the disease.

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that is crucial for various physiological processes, encompassing the normal functioning of the nervous system, as well as the development and maturation of red blood cells. It boasts antioxidant properties, serves as a cofactor in mitochondrial energy metabolism, and contributes significantly to DNA synthesis, the methylation cycle, and epigenetic regulation (16). Vitamin B12 is a redox-active compound containing a cobalt atom that cycles between oxidation states. The removal of superoxide induces its oxidation, rendering methionine synthase and methylmalonyl-CoA mutase inactive, thereby disrupting gene expression and energy production. Clinically, high doses of vitamin B12 can be utilized to reduce oxidative stress and maintain cofactor function (17). Moreover, vitamin B12 deficiency is associated with numerous hallmarks of aging at both cellular and organismal levels, triggering metabolic abnormalities that lead to heightened DNA damage, increased mitochondrial dysfunction, and disruptions in epigenetic regulation, ultimately contributing to cellular senescence (18). These functions play a crucial role in arthritis. Given that the body stores approximately 1 to 5 milligrams of vitamin B12, symptoms of vitamin B12 deficiency may take several years to manifest. Vitamin B12 deficiency can mimic a wide

range of illnesses, presenting diverse perspectives from hematologists, neurologists, gastroenterologists, general practitioners, or nutritionists. Enhanced vigilance among physicians and raised awareness among patients are often the reasons for its early detection, sometimes preceding the detection of deficiencies before the primary onset of disease during a particular stage (19). Food sources of vitamin B12 encompass animal-based products like meat, fish, poultry, eggs, and dairy. Plant-based foods do not naturally contain vitamin B12, making vegetarians and strict vegans who abstain from animal products more susceptible to vitamin B12 deficiency (20). The toxicity potential of vitamin B12 is low, making it generally considered safe even in high doses, as the body does not store excess amounts. However, this study found through MR analysis that excessive vitamin B12 may promote the incidence of monoarthritis and other arthritis, and further experimental validation is needed.

Some other MNs, such as folic acid, selenium, and vitamin E, although not indicating a causal relationship with arthritis in MR analysis, showed positive results in NHANES data analysis. The RCS analysis found that when these micronutrients were supplemented to certain doses in the diet, significant nonlinear relationships with the incidence of arthritis were observed at the following doses: vitamin E (10.92 μg), folic acid (119 μg), and selenium (101.0 mg). This indicates that at these dose levels, these micronutrients may influence the risk of arthritis onset. However, beyond these thresholds, further increases in dosage did not result in additional protective effects. These thresholds, however, do not represent the recommended optimal intake levels. For instance, the observed threshold for selenium (101.0 mg) is far higher than its DRI value (about 55 μg/day). This difference may arise because the DRI value is based on extensive research aimed at meeting the health needs of the general population, while the RCS model reflects the sensitivity of specific health indicators (such as arthritis risk) and may be influenced

by confounding factors (such as diet patterns, lifestyle, and genetic factors), as well as differences between experimental conditions and real-life scenarios. Therefore, these results should be interpreted with caution and considered as references only, while actual intake recommendations need to be evaluated in conjunction with the DRI. Nevertheless, this study provides new insights into the potential role of micronutrients in the prevention of arthritis. Folic acid (vitamin B9) is a water-soluble vitamin that promotes red blood cell production, prevents fetal neural tube defects, maintains cardiovascular health, and enhances immunity. It also participates in DNA synthesis and repair, helping to prevent dementia and neural tube defects (21). One study indicated that folic supplementation can reduce serum triglyceride and total cholesterol levels (22). In arthritis, FA-targeted aminopterin (AMT) therapy can modulate macrophage function and effectively treat inflammatory animal models, with targeting folate receptor β on macrophages providing rapid relief from inflammation (23). Vitamin E is a fat-soluble vitamin that acts mainly through its antioxidant and anti-inflammatory properties in arthritis treatment (24). It can reduce oxidative stress, protect chondrocytes, and lower levels of inflammation-related cytokines, thereby alleviating arthritis symptoms (25). Selenium is an essential trace element for the human body, protecting cells from oxidative damage through its antioxidant effects, promoting the proliferation and differentiation of chondroprogenitor cells, inhibiting the degradation of chondrocytes and the matrix, and participating in DNA damage repair (26). Additionally, selenium's anti-inflammatory properties also help reduce arthritis pain and inflammation (27).

Although each micronutrient possesses unique functions and effects, their impact on arthritis predominantly revolves around antioxidant, anti-inflammatory, and cartilage health-promoting properties. Furthermore, MNs do not work in isolation; rather, they collaborate through various combinations to enhance their efficacy. For instance,

vitamin D and calcium supplements are commonly used together to optimize bone health (28). Selenium and vitamin E exhibit antioxidant properties, assisting in reducing oxidative stress and inflammation (29). The optimization of these micronutrient combinations and the identification of optimal dosages for use remain avenues for further research.

This study investigated the association between MNs and arthritis through a joint analysis of the NHANES and GWAS databases. Although meaningful results were obtained, several limitations exist. First, NHANES is a cross-sectional survey project, with all data collected at a single time point, which restricts our ability to fully infer the causal relationship between MNs and arthritis. Second, the diagnosis of arthritis was based on self-reports from questionnaires, which may lack precision and reliability, underestimate the prevalence of the disease, and pose a risk of misclassification bias. Moreover, the heterogeneity of arthritis subtypes may further dilute true associations or increase the risk of false-positive results. Third, this study selected 14 MNs for analysis, based on the availability of corresponding data in both the NHANES and GWAS databases. Although some MNs (such as zinc) are known to be associated with arthritis, they were not included in this study due to limitations in the availability of GWAS data. This may lead to selection bias and limit our comprehensive understanding of the roles of these key MNs. Fourth, although we controlled for many covariates, residual and unmeasured confounding factors may still exist, given the related confounding factors. Finally, the GWAS data were derived from European populations, while the NHANES dataset includes multiple ethnicities. This population mismatch may limit the external validity of the MR analysis results and restrict our ability to generalize our findings to other ethnic groups. To overcome these limitations, future studies could consider the following directions: 1) using multi-ethnic GWAS datasets to conduct more representative Mendelian randomization analyses to enhance the

external validity of the results; 2) including more MNs known to be associated with arthritis to more comprehensively assess the relationship between diet and arthritis; 3) adopting a longitudinal study design to better infer causal relationships; 4) combining clinical diagnoses and biomarker data to improve the accuracy and reliability of arthritis diagnoses. Additionally, further research into the interactions between different MNs and their mechanisms of action in different arthritis subtypes will also help optimize prevention and treatment strategies.

CONCLUSIONS

In conclusion, through the joint analysis of NHANES and MR, a variety of MNs in the diet were found to be closely related to arthritis, and a comprehensive analysis of many aspects was carried out to obtain some meaningful conclusions, but the underlying mechanism needs to be further clarified.

REFERENCES

1. Song P, Cui Z, Hu L. Applications and prospects of intra-articular drug delivery system in arthritis therapeutics. J Control Release 2022;352:946-60. DOI: 10.1016/j.jconrel.2022.11.018
2. Duca LM, Helmick CG, Barbour KE, Guglielmo D, Murphy LB, Boring MA, et al. Self-Management Education Class Attendance and Health Care Provider Counseling for Physical Activity Among Adults with Arthritis - United States, 2019. MMWR Morb Mortal Wkly Rep 2021;70:1466-71. DOI: 10.15585/mmwr.mm7042a2
3. Berger MM, Shenkin A, Schweinlin A, Amrein K, Augsburger M, Biesalski H-K, et al. ESPEN micronutrient guideline. Clin Nutr 2022;41:1357-424. DOI: 10.1016/j.clnu.2022.02.015
4. Bañuls-Mirete M, Ogdie A, Guma M. Micronutrients: Essential Treatment for Inflammatory Arthritis? Curr Rheumatol Rep 2020;22:87. DOI: 10.1007/s11926-020-00962-z
5. Farsijani S, Mao Z, Cauley JA, Newman AB. Comprehensive assessment of chrononutrition behaviors among nationally representative adults: Insights from National Health and Nutrition Examination Survey (NHANES) data. Clin Nutr 2023;42:1910-21. DOI: 10.1016/j.clnu.2023.08.007
6. Wessels I, Rink L. Micronutrients in autoimmune diseases: possible therapeutic benefits of zinc and vitamin D. J Nutr Biochem 2020;77:108240. DOI: 10.1016/j.jnutbio.2019.108240
7. Yin Y, Dong Y, Cao Y, Dong G. Association of Vitamin E Intake with All-Cause Mortality Among Individuals with Rheumatoid Arthritis: A Cohort Study from the NHANES 1999-2018. J Am Nutr Assoc 2024;1-7. DOI: 10.1080/27697061.2024.2401055
8. Lin Z, Pan W. A robust cis-Mendelian randomization method with application to drug target discovery. Nat Commun 2024;15:6072. DOI: 10.1038/s41467-024-50385-y

9. Hu X, Cai M, Xiao J, Wan X, Wang Z, Zhao H, et al. Benchmarking Mendelian randomization methods for causal inference using genome-wide association study summary statistics. Am J Hum Genet 2024;111:1717-35. DOI: 10.1016/j.ajhg.2024.06.016
10. Kim JY, Song M, Kim MS, Natarajan P, Do R, Myung W, et al. An atlas of associations between 14 micronutrients and 22 cancer outcomes: Mendelian randomization analyses. BMC Med 2023;21:316. DOI: 10.1186/s12916-023-03018-y
11. Qiu Y, Li C, Huang Y, Wu C, Li F, Zhang X, et al. Exploring the causal associations of micronutrients on urate levels and the risk of gout: A Mendelian randomization study. Clin Nutr 2024;43:1001-12. DOI: 10.1016/j.clnu.2024.03.003
12. Rizzoli R, Biver E, Brennan-Speranza TC. Nutritional intake and bone health. Lancet Diabetes Endocrinol 2021;9:606-21. DOI: 10.1016/S2213-8587(21)00119-4
13. Warensjö Lemming E, Byberg L, Höijer J, Larsson SC, Wolk A, Michaëlsson K. Combinations of dietary calcium intake and mediterranean-style diet on risk of hip fracture: A longitudinal cohort study of 82,000 women and men. Clin Nutr 2021;40:4161-70. DOI: 10.1016/j.clnu.2021.01.043
14. Yu Z, Li Y, Ba DM, Veldheer SJ, Sun L, Geng T, et al. Trends in Calcium Intake among the US Population: Results from the NHANES (1999–2018). Nutrients 2024;16:726. DOI: 10.3390/nu16050726
15. Cracknell RO, Tavassoli T, Field DT. High-dose Vitamin-B6 reduces sensory over-responsivity. J Psychopharmacol 2024;38(12):1147-56. DOI: 10.1177/02698811241271972
16. Wolffenbuttel BH, Owen PJ, Ward M, Green R. Vitamin B12. BMJ 2023;383:e071725. DOI: 10.1136/bmj-2022-071725
17. Offringa AK, Bourgonje AR, Schrier MS, Deth RC, van Goor H. Clinical implications of vitamin B12 as redox-active cofactor.

Trends Mol Med 2021;27:931-4. DOI: 10.1016/j.molmed.2021.07.002

18. Simonenko SY, Bogdanova DA, Kuldyushev NA. Emerging Roles of Vitamin B12 in Aging and Inflammation. Int J Mol Sci 2024;25:5044. DOI: 10.3390/ijms25095044
19. Sobczyńska-Malefora A, Delvin E, McCaddon A, Ahmadi KR, Harrington DJ. Vitamin B12 status in health and disease: a critical review. Diagnosis of deficiency and insufficiency - clinical and laboratory pitfalls. Crit Rev Clin Lab Sci 2021;58:399-429. DOI: 10.1080/10408363.2021.1885339
20. Silverstein WK, Cheung MC, Lin Y. Vitamin B12 deficiency. CMAJ 2022;194:E843. DOI: 10.1503/cmaj.220306
21. Elizabeth KE. Folate: Its Biological Interactions and Strategies to Achieve Sufficiency Without Causing Excess. Am J Public Health 2021;111:347-9. DOI: 10.2105/AJPH.2020.306067
22. Asbaghi O, Ashtary-Larky D, Bagheri R, Nazarian B, Pourmirzaei Olyaei H, Rezaei Kelishadi M, et al. Beneficial effects of folic acid supplementation on lipid markers in adults: A GRADE-assessed systematic review and dose-response meta-analysis of data from 21,787 participants in 34 randomized controlled trials. Crit Rev Food Sci Nutr 2022; 62:8435-53. DOI: 10.1080/10408398.2021.1928598
23. Lu YJ, Wheeler LW, Chu H, Kleindl PJ, Pugh M, You F, et al. Targeting folate receptor beta on monocytes/macrophages renders rapid inflammation resolution independent of root causes. Cell Rep Med 2021;2:100422. DOI: 10.1016/j.xcrm.2021.100422
24. Zaaboul F, Liu Y. Vitamin E in foodstuff: Nutritional, analytical, and food technology aspects. Compr Rev Food Sci Food Saf 2022;21:964-98. DOI: 10.1111/1541-4337.12924
25. Asbaghi O, Sadeghian M, Nazarian B, Sarreshtedari M, Mozaffari-Khosravi H, Maleki V, et al. The effect of vitamin E

supplementation on selected inflammatory biomarkers in adults: a systematic review and meta-analysis of randomized clinical trials. Sci Rep 2020;10:17234. DOI: 10.1038/s41598-020-73741-6

26. Kang D, Lee J, Wu C, Guo X, Lee BJ, Chun J-S, et al. The role of selenium metabolism and selenoproteins in cartilage homeostasis and arthropathies. Exp Mol Med 2020;52:1198-208. DOI: 10.1038/s12276-020-0408-y

27. Zheng C, Wu A, Zhai X, Ji H, Chen Z, Chen X, et al. The cellular immunotherapy of integrated photothermal anti-oxidation Pd-Se nanoparticles in inhibition of the macrophage inflammatory response in rheumatoid arthritis. Acta Pharm Sin B 2021;11:1993-2003. DOI: 10.1016/j.apsb.2021.02.021

28. Michos ED, Cainzos-Achirica M, Heravi AS, Appel LJ. Vitamin D, Calcium Supplements, and Implications for Cardiovascular Health: JACC Focus Seminar. J Am Coll Cardiol 2021;77:437-49. DOI: 10.1016/j.jacc.2020.09.617

29. Panda SK, Peng V, Sudan R, Ulezko Antonova A, Di Luccia B, Ohara TE, et al. Repression of the aryl-hydrocarbon receptor prevents oxidative stress and ferroptosis of intestinal intraepithelial lymphocytes. Immunity 2023;56:797-812.e4. DOI: 10.1016/j.immuni.2023.01.023

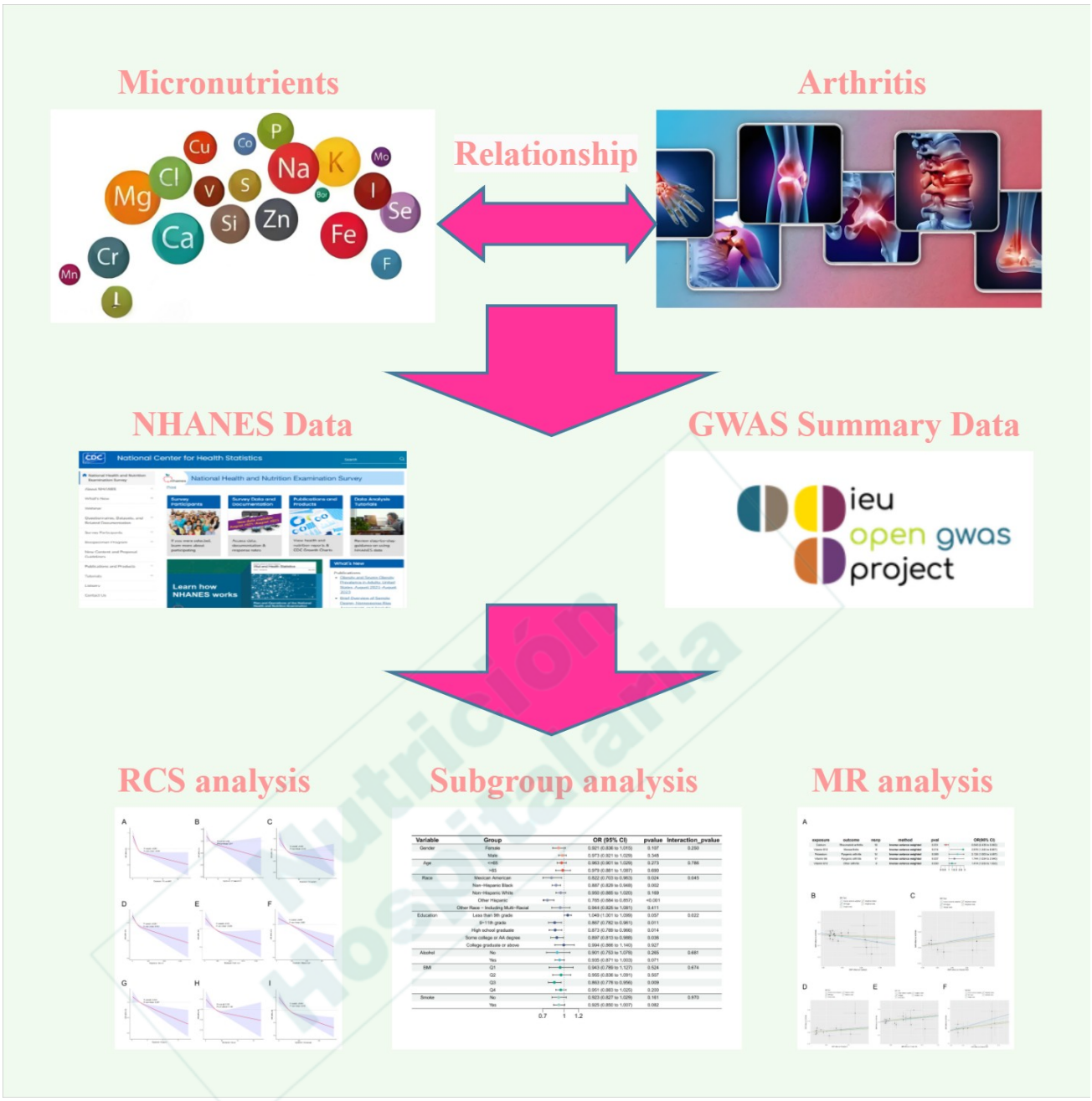

Figure 1. Overview of the study.

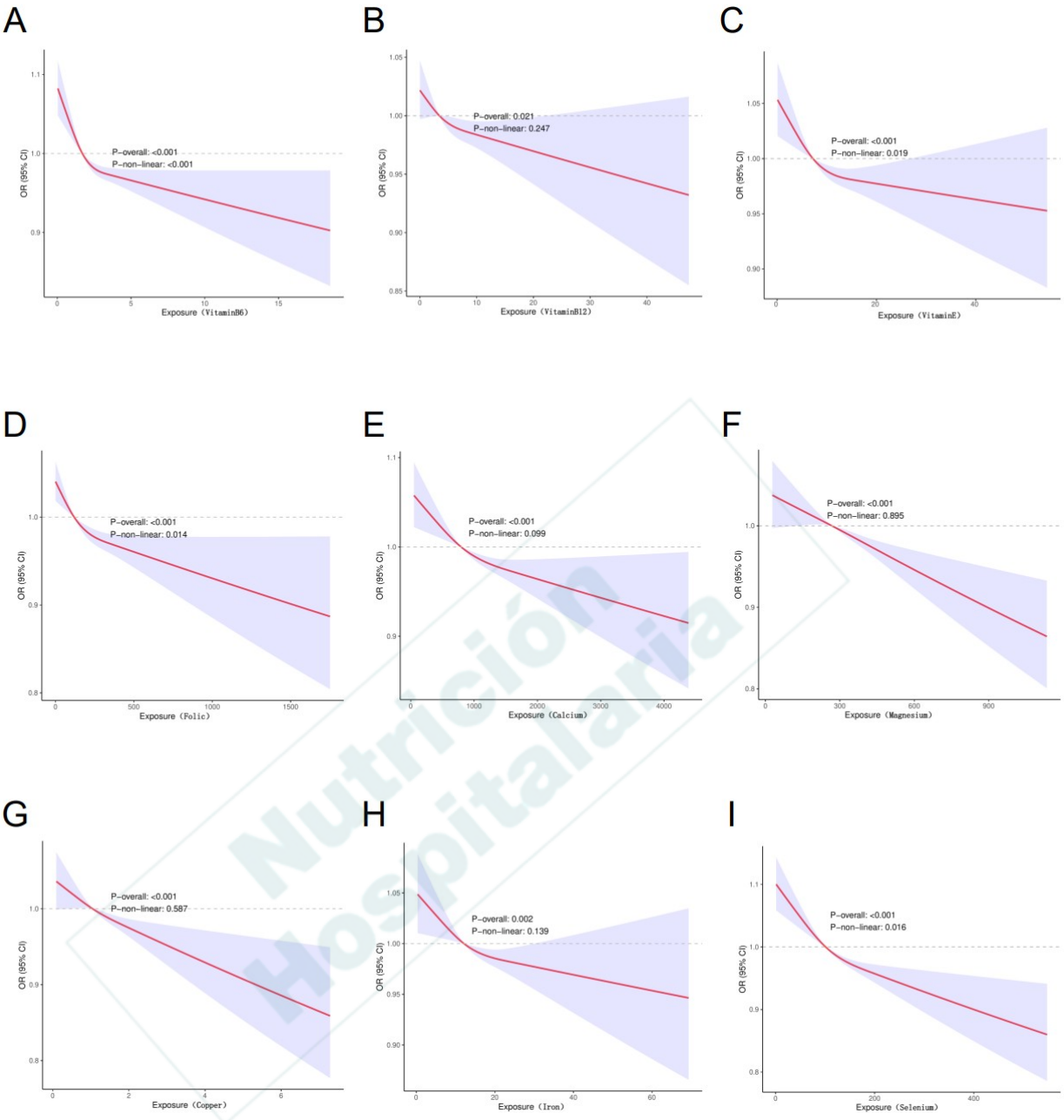

Figure 2. A-I Restricted cubic spline plot of the association between micronutrients levels in arthritis risk.

Variable	Group	OR (95% CI)	pvalue	Interaction_pvalue
Gender	Female	0.921 (0.836 to 1.015)	0.107	0.250
	Male	0.973 (0.921 to 1.029)	0.348	
Age	<=65	0.963 (0.901 to 1.029)	0.273	0.786
	>65	0.979 (0.881 to 1.087)	0.690	
Race	Mexican American	0.822 (0.703 to 0.963)	0.024	0.045
	Non−Hispanic Black	0.887 (0.829 to 0.948)	0.002	
	Non−Hispanic White	0.950 (0.885 to 1.020)	0.169	
	Other Hispanic	0.765 (0.684 to 0.857)	<0.001	
	Other Race − Including Multi−Racial	0.944 (0.825 to 1.081)	0.411	
Education	Less than 9th grade	1.049 (1.001 to 1.099)	0.057	0.022
	9−11th grade	0.867 (0.782 to 0.961)	0.011	
	High school graduate	0.873 (0.789 to 0.966)	0.014	
	Some college or AA degree	0.897 (0.813 to 0.988)	0.036	
	College graduate or above	0.994 (0.866 to 1.140)	0.927	
Alcohol	No	0.901 (0.753 to 1.078)	0.265	0.681
	Yes	0.935 (0.871 to 1.003)	0.071	
BMI	Q1	0.943 (0.789 to 1.127)	0.524	0.674
	Q2	0.955 (0.836 to 1.091)	0.507	
	Q3	0.863 (0.778 to 0.956)	0.009	
	Q4	0.951 (0.883 to 1.025)	0.200	
Smoke	No	0.923 (0.827 to 1.029)	0.161	0.970
	Yes	0.925 (0.850 to 1.007)	0.082	

Figure 3. Subgroup analysis of forest maps (BMI: body mass index; Q1: 14.5-24.8; Q2: 24.8-28.6; Q3: 28.6-33.5; Q4: 33.5-84.4; CI: confidence interval; OR: odds ratio).

A

exposure	outcome	nsnp	method	pval	OR(95% CI)
Calcium	Rheumatoid arthritis	19	**Inverse variance weighted**	0.031	0.648 (0.436 to 0.962)
Vitamin B12	Monoarthritis	8	**Inverse variance weighted**	0.014	2.876 (1.240 to 6.667)
Potassium	Pyogenic arthritis	14	**Inverse variance weighted**	0.049	2.124 (1.003 to 4.497)
Vitamin B6	Pyogenic arthritis	17	**Inverse variance weighted**	0.037	1.744 (1.034 to 2.940)
Vitamin B12	Other arthritis	8	**Inverse variance weighted**	0.030	1.414 (1.035 to 1.932)

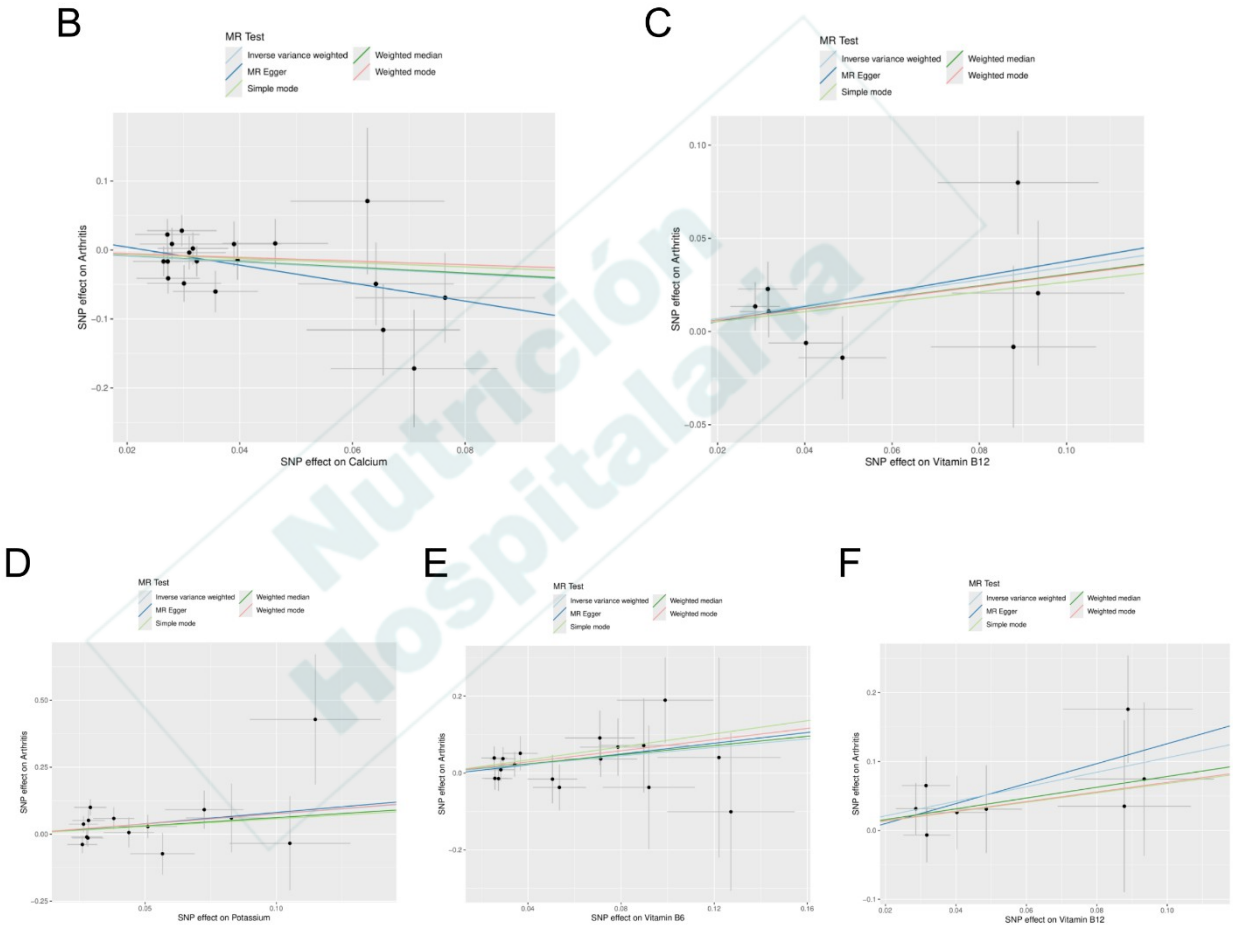

Figure 4. A. Forest map of micronutrients for arthritis. B. Scatter plots of calcium and rheumatoid arthritis. C. Scatter plots of vitamin B12 and monoarthritis. D. Scatter plots of potassium and pyogenic arthritis. E. Scatter plots of vitamin B6 and pyogenic arthritis. F. Scatter plots of vitamin B12 and other arthritis.

Table I. Baseline characteristics of arthritic and non-arthritic groups

Characteristic	**Level**	**Overall**	**Non-arthritis**	**Arthritis**	***p*-value**
n		9289	6596	2693	
Gender (%)	Female	4732 (50.9)	3172 (48.1)	1560 (57.9)	< 0.001
	Male	4557 (49.1)	3424 (51.9)	1133 (42.1)	
Age		50.53 (17. 62)	45.80 (16.9 4)	62.13 (13. 36)	< 0.001
Race (%)	Mexican American	1435 (15.4)	1134 (17.2)	301 (11.2)	< 0.001
	Non-Hispanic black	2066 (22.2)	1459 (22.1)	607 (22.5)	
	Non-Hispanic white	3295 (35.5)	2084 (31.6)	1211 (45.0)	
	Other Hispanic	1039 (11.2)	750 (11.4)	289 (10.7)	
	Other race - including multi-Racial	1454 (15.7)	1169 (17.7)	285 (10.6)	
Education (%)	9-11th grade	1051 (11.3)	727 (11.0)	324 (12.0)	0.007
	College graduate or above	2287 (24.6)	1763 (26.7)	524 (19.5)	
	High school graduate	2160 (23.3)	1499 (22.7)	661 (24.5)	

	Less than 9th grade	869 (9.4)	579 (8.8)	290 (10.8)	
	Some college or AA degree	2922 (31.5)	2028 (30.7)	894 (33.2)	
Alcohol (%)	No	1947 (21.0)	1398 (21.2)	549 (20.4)	0.977
	Yes	7342 (79.0)	5198 (78.8)	2144 (79.6)	
BMI		29.82 (7.2 3)	29.11 (6.92)	31.56 (7.6 7)	< 0.001
Smoking (%)	No	5300 (57.1)	4024 (61.0)	1276 (47.4)	< 0.001
	Yes	3989 (42.9)	2572 (39.0)	1417 (52.6)	

Data were presented as median (interquartile range) or *n* (%).

Table II. Baseline characteristics of 14 trace elements with arthritis and non-arthritis groups

Characteristic	**Overall**	**Non- arthritis**	**Arthritis**	***p*-value**
Vitamin E	8.77 (6.79)	8.91 (6.92)	8.42 (6.42)	0.013
Vitamin A	603.72 (607.13)	606.11 (623.76)	597.85 (564.39)	0.979
Carotene	381.57 (1153.73)	386.94 (1182.76)	368.44 (1079.43)	0.855
VitaminB6	2.07 (1.85)	2.15 (1.90)	1.88 (1.73)	0.008
Folic acid	166.02 (182.74)	172.18 (193.51)	150.93 (152.21)	0.005
Vitamin B12	4.67 (5.47)	4.82 (5.85)	4.31 (4.35)	0.016
Vitamin C	79.57 (93.28)	81.07 (96.73)	75.89 (84.14)	0.463
Vitamin D	4.39 (5.67)	4.43 (5.74)	4.29 (5.50)	0.446
Calcium	909.48 (572.08)	930.92 (590.05)	856.95 (521.88)	0.006
Magnesium	295.80 (150.76)	302.22 (154.44)	280.09 (140.12)	0.002
Iron	13.87 (8.47)	14.10 (8.54)	13.32 (8.26)	0.042
Copper	1.19 (0.83)	1.22 (0.89)	1.11 (0.63)	0.006
Potassium	2560.14(1253.83)	2590.42 (1272.15)	2485.95 (1204.81)	0.278
Selenium	113.66 (66.79)	117.29 (68.74)	104.77 (60.85)	< 0.001

Table III. Details of sensitivity analysis of MR Results of micronutrients and arthritis

Exposure	**Outcome**	**Heterogeneity tests MR-Egger/IVW Cochran's Q (*p*-value)**	**Directional horizontal pleiotropy test *p*-value**	**MR-PRESSO results Global test/*p*-value**
Calcium	Rheumatoid arthritis	18.46(0.360)/19.972 (0.334)	0.254	22.04/0.37
Vitamin B12	Monoarthritis	2.98 (0.811)/3.16 (0.869)	0.685	4.501/0.844
Potassium	Pyogenic arthritis	18.886 (0.091)/18.898 (0.126)	0.931	21.999/0.127
Vitamin B6	Pyogenic arthritis	8.351 (0.908)/8.418 (0.935)	0.798	9.384/0.949
Vitamin B12	Other arthritis	7.846 (0.249)/7.877 (0.343)	0.881	11.213/0.339